DEVELOPMENT OF THE OVULATE STROBILUS AND YOUNG OVULE OF ZAMIA FLORIDANA

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(WITH TWENTY-TWO FIGURES)

The results of a study of the development of the staminate strobilus and microsporangia of *Zamia* were published by the writer in 1907 (1). Some of the material, sent from Miami, Florida, in June of the years 1905 and 1906 for this investigation, included ovulate cones. Later in the year 1906 an effort was made to secure a complete series of young ovulate cones, for *Zamia*, alone of the cycads of North America, exists in such profusion that whole plants may be sacrificed to secure a single small cone from each. There are some stages not yet covered by the series of cones which has been obtained, but it seems worth while at this point in the study of the material to sum up the results, and to postpone conclusions from these results and their theoretical bearing upon other cycad studies until a complete series has been gathered.

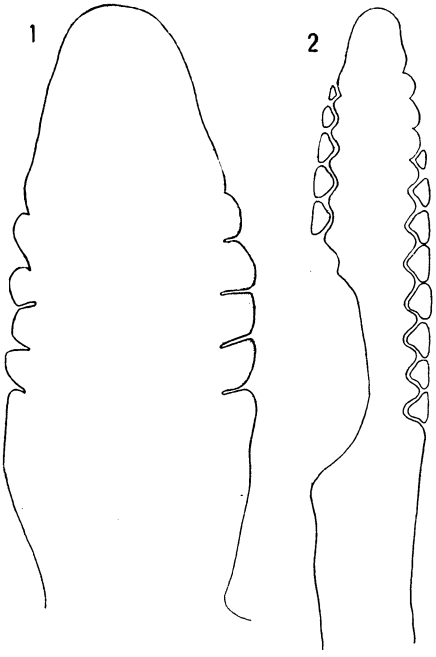
Each year, since 1906, an attempt has been made to secure material which should give the origin of the integument. In 1907, out of eight or ten plants sent from Florida between July 25 and August 8, not one contained an ovulate cone. I do not know whether this was an unfruitful year or whether the collector was unfortunate in the plants he gathered. Another year, knowing just the period during which the material ought to be gathered, careful collections were made, but in every case the cones had reached a development two weeks ahead of that of the previous year, so that it is evident collections of *Zamia* must be made often and during long periods in order to obtain a full series.

The facts ascertained from the material cover the period from the appearance of the ovulate cone to the time when the developing endosperm has partially filled the embryo sac, and will be treated under three periods of growth.

1. Development of the strobilus and the sporophylls

Plants gathered early in June show a slight elevation which elongates to form the strobilus, but at this stage it is impossible to distinguish the ovulate strobili from the staminate. As stated in my description of the staminate plants (1), the strobili are deeply sunken in the tip of the crown and are completely covered by the bases of the rosette of leaves.

A strobilus of July 5 is about 5 mm. long, and at this time can be recognized as ovulate. There are defined in a median longitudinal section five sporophylls on a side (fig. 1). A comparison with a staminate cone of July 8, with eleven sporophylls on a side, gives approximately the same length, but greater breadth and larger sporophylls than those of the staminate cone (fig. 2).



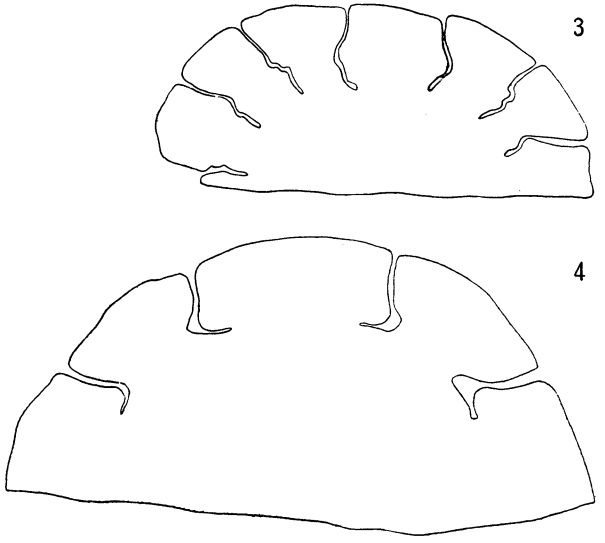
FIGS. 1, 2.—Fig. 1, longitudinal section of young ovulate strobilus, showing sporophylls (July 5). ×40; fig. 2, longitudinal section of young staminate strobilus, showing sporophylls (July 8). ×40.

In comparing cross-sections of strobili of July 25, much the same points are noticed. A staminate strobilus (fig. 3) has on an average fourteen to sixteen sporophylls on a cross-section, while the ovulate strobilus (fig. 4) has seldom more than ten. A difference in the size of the sporophylls as well as of the strobili is seen also.

The development of the sporophylls is identical in the two strobili, at least in these early stages, except that the elevation representing the sporophyll in the ovulate strobilus is broader and includes more cells in a hypodermal position which are dividing periclinally. As the sporangia appear, there are fewer meristematic points on the ovulate

sporophylls, as shown in figs. 3 and 4. The lobes themselves are broader and the cells of the inner surface show a deeper stain, indicating that this is the region where active growth is taking place. This region is included by the dotted line drawn on one of the sporophylls in fig. 4.

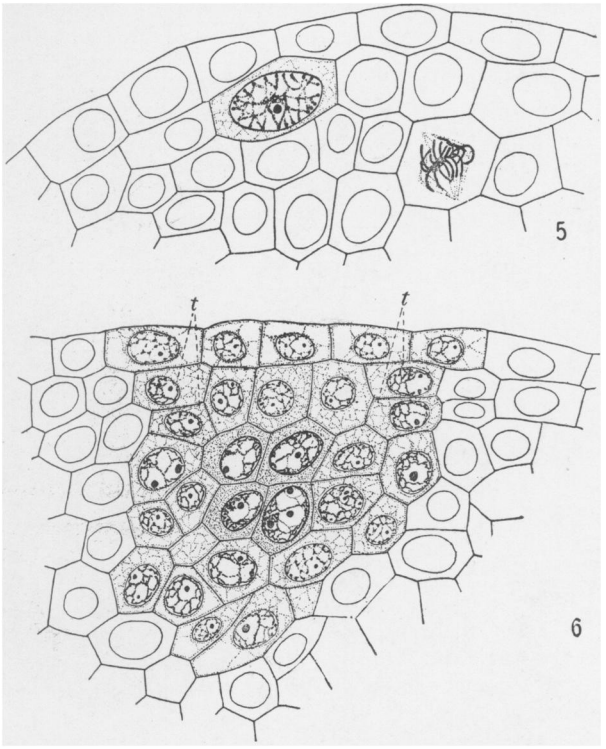
In a megasporophyll of July 25, of the size in fig. 4, a cell can be distinguished for the first time from those about it by its size and



FIGS. 3, 4.—Fig. 3, transverse section of staminate strobilus, showing sporophylls and position of sporangia (July 26). ×40; fig. 4, transverse section of ovulate strobilus (July 26), showing sporophylls and meristematic group of cells on one lobe indicated by dotted line. ×40.

by its larger nucleus with deeply staining chromatin. This cell is hypodermal in origin (fig. 5) and resembles the single archesporial cell in the staminate sporangium. About ten or twelve cells in a cross-section form a meristematic group, some of which are actively dividing, but this archesporial cell is easily distinguished from these. In another sporophyll of July 25, but evidently one a little more developed as shown by its increased size, the development of this group of cells has gone on still farther, and now four cells in cross-section can be clearly distinguished from the others (fig. 6). This group is separated from the epidermis by one layer of cells, and the

size of its nuclei and the position of its walls suggest an origin from a single archesporial cell. It resembles, too, the group in the staminate strobilus which gives rise to the sporogenous mass in the microsporangium. Two pairs of cells (*t*) just under the epidermis, from the



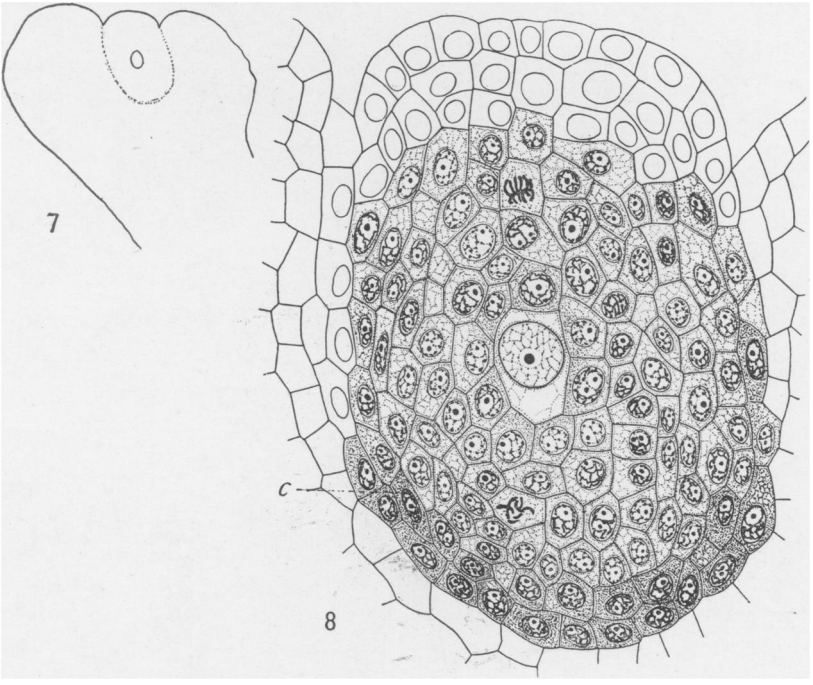
FIGS. 5, 6.—Fig. 5, detail of sporophyll of fig. 4, showing hypodermal archesporial cell. ×40; fig. 6, more advanced stage, showing group of meristematic cells (*t*) which may give rise to integument. ×1400.

direction of their walls indicate that they have arisen from periclinal divisions of hypodermal cells, and suggest the first divisions causing the elevation of the integument.

Just at this point there is the break in the continuity of the series referred to, and the strobili of August 8, which is the date of the next collection, show on either lobe of the sporophyll the projecting nucellus and the integument, which is only slightly elevated at this time.

2. Development of the ovule

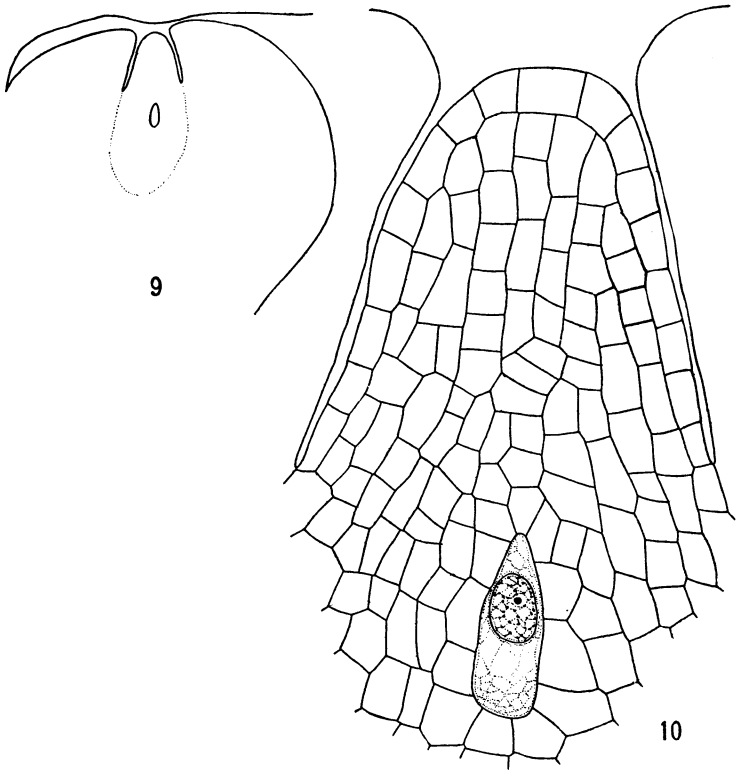
The ovule of August 8 shown in figs. 7 and 8 is younger, I think, than that of *Stangeria* as figured by LANG (**2**), and about the same age as that of *Ceratozamia* shown by TREUB (**3**). The megaspore mother cell is easily picked out at this stage as a larger cell, which is



FIGS. 7, 8.—Fig. 7, young ovule (August 8), showing (by dotted line) group of cells arising from archesporium. ×136; fig. 8, detail of fig. 7, showing megaspore mother cell, adjacent tissue dividing, and flattened cells (*c*) bounding nucellus. ×930.

more vacuolated and which has a large nucleus with the chromatin arranged in a fine network. The cells about it stain rather more deeply, and toward the chalazal end there are several rows of flattened cells (*c*) which stain still more deeply, and which form a boundary to the ovule in this direction. These rows curve up about the "sporogenous group" and meet the epidermis at the point where the integument and nucellus are separated from each other.

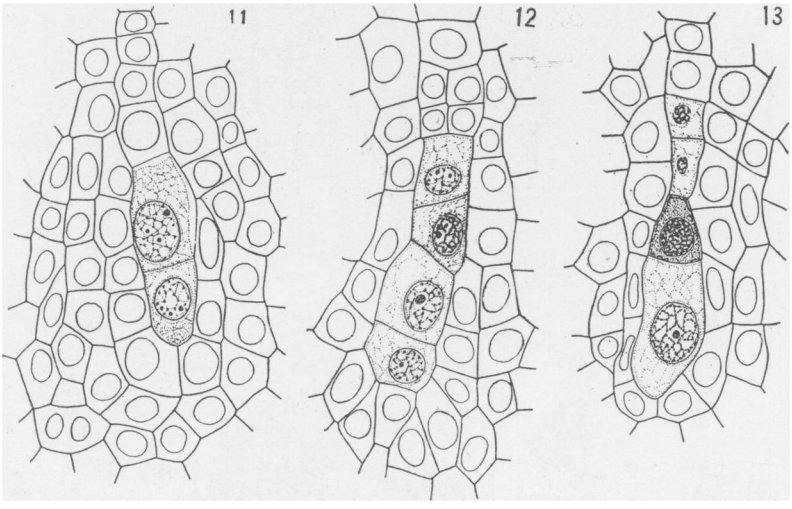
In the region of the nucellar apex, the cells are constantly dividing as the nucellus increases, but this cell activity is not confined to that part of the ovule. On either side and below the mother cell there is constant division, as is shown in sections through the mother cell



FIGS. 9, 10.—Fig. 9, older ovule, showing megaspore mother cell. ×136; fig. 10, detail of fig. 9, showing large increase in nucellar apex. ×930.

and in sections adjacent to it. Often there are three spindles in a section 8 μ thick. This megaspore mother cell enlarges somewhat before dividing again, while the nucellus by repeated periclinal divisions (figs. 9, 10) is becoming extended. This changes the broad, flat outline of the projecting portion to a narrower, more pointed one. The integument keeps pace in its development and narrows

the micropyle by growing in the direction of the nucellus. During this change in the nucellus the mother cell divides (fig. 11) into an upper larger and a lower smaller cell. The next stage seen (fig. 12) had a row of four megaspores, and the position of the upper two and again of the lower two would indicate that they were derived from these first two cells by a further division. Fig. 13 shows a disappearance of the two upper cells, indicated by the small nuclei and the

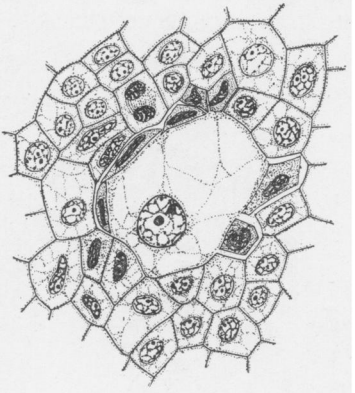


FIGS. 11, 12, 13.—Fig. 11, two cells arising from megaspore mother cell; fig. 12, chain of four megaspores. ×930; fig. 13, four megaspores: three degenerating, the fourth the embryo sac. ×930.

narrowed effect of the cells themselves. The nucleus of the cell just over the basal one of the chain is being flattened and crushed by the development of its sister cell, which is to be the embryo sac. In other sections there is a deeply stained cap over the micropylar end of the embryo sac, which is probably the remnant of the other megaspore or megaspores; at last this also disappears. From this time on the embryo sac enlarges rapidly and becomes more vacuolated (figs. 10, 14).

3. Development of the female gametophyte and changes in the "spongy tissue"

Many slides show the uninucleate condition of the embryo sac, indicating that this stage is quite prolonged. Fig. 14 shows a few cells surrounding the embryo sac, which are broken down by the growing sac; material killed August 29 is about the first to show this condition. The nucleus of the embryo sac divides as is described for other species, and the nuclei take the polar position. There is a slight indication of cytoplasm forming a lining to the wall, but the karyokinetic figure in the section drawn must have formed a little to the



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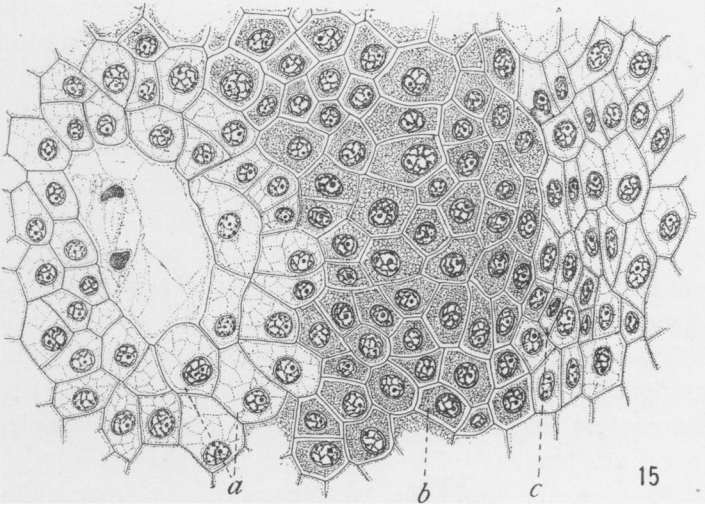

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FIGS. 14, 15.—Fig. 14, detail of enlarging embryo sac. ×930; fig. 15, detail of embryo sac and "spongy tissue" (August 29): *a*, active nutritive cells; *b*, tissue of closely packed cells; *c*, flattened cells bounding group. ×930.

side of the middle of the sac (fig. 15). Fig. 15 shows the sac and the surrounding cells on one side as far as the flattened rows (*c*)

forming the boundary of the group of cells which we may call the "spongy tissue," using STRASBURGER'S term. These flattened cells of the periphery were first seen in the young ovule (fig. 8). The "spongy tissue" may perhaps be referred to the mass which originated in a single archesporial cell, but the lack of sufficient material prevents me from drawing conclusions.

By the time the embryo sac nucleus has divided, the "spongy tissue" shows differentiation. Next to the embryo sac membrane the cells (*a*) are larger, more vacuolated, and often bulge into the sac as pressure is released on this side. Occasionally these cells are broken apart and the walls are very indistinct. The next rows (*b*), often eight or ten in number, are made up of smaller cells, therefore seeming to be closely packed and often dividing in such planes that they form rows radiating from the center of the sac. The nuclei and cells themselves stain deeply, contrasting both with the inner layers mentioned and with the flattened peripheral rows (*c*). Quite often a spindle is seen in these cells, so that this is by no means a degenerating tissue. The cytoplasm in the preparations has separated from the walls a little, and the walls are "so transparent that the nuclei seem to be floating in cytoplasm" (3), but I am convinced that this is due to a slow passage of the killing fluid into the tissue and the consequent shrinkage. Fig. 16 shows the two embryo sac nuclei at the poles, and fig. 17 has four nuclei in one section, one of which has not yet passed to the peripheral position. The interior of the sac in these sections has a beautiful vacuolate structure, and the "spongy tissue" has not changed appreciably.

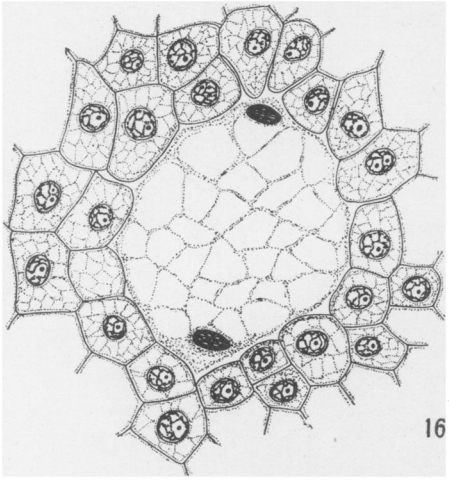
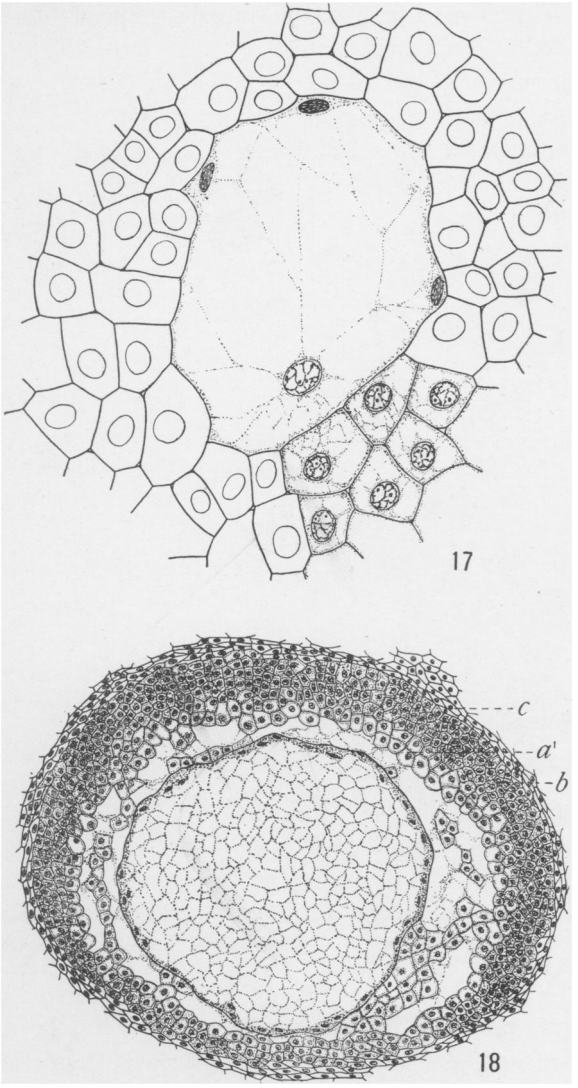


FIG. 16.—Polar position of embryo sac nuclei. ×930.

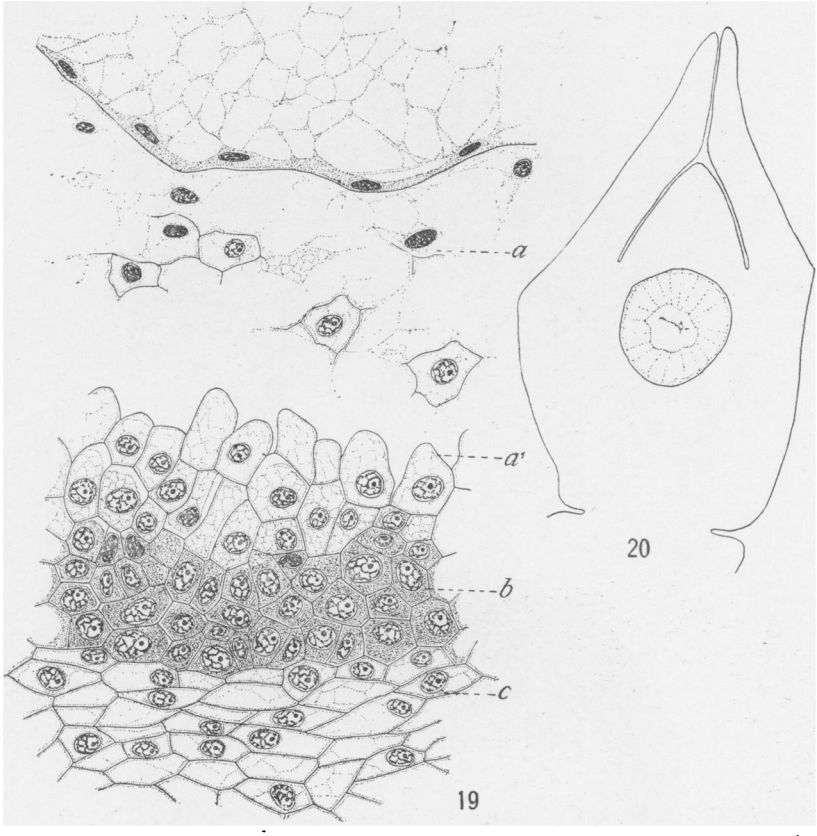
The next series of slides obtained give further divisions of free nuclei and their peripheral position in the cytoplasmic lining, which



FIGS. 17, 18.—Fig. 17, four nuclei of embryo sac. ×930; fig. 18, embryo sac with layer of cells; spongy tissue beginning to break down. ×250.

is now a little thicker. The cytoplasm here is quite foamlike in its structure (figs. 18, 19). Fig. 19 is a detail of the sac and the "spongy

tissue" at the micropylar end. The result of the growth of the sac is seen in the breaking down of the "spongy tissue"; the layers nearest the sac are almost used up (*a*). Seemingly the wall of each cell is



FIGS. 19, 20.—Fig. 19, detail of fig. 18: *a*, "spongy tissue" broken down; *a'*, nutritive tissue; *b*, compact tissue; *c*, flattened cells. ×930; fig. 20, ovule showing unusual development of "spongy tissue." ×37.

attacked first, probably by an enzyme secreted by the developing gametophyte, and the cells become separated from each other. Sometimes a nucleus has its position in an isolated cell, and sometimes it is surrounded by cytoplasm. The breaking down of this tissue resembles that described by CHAMBERLAIN (**4**) in the formation of the pollen chamber of *Dioon edule*.

Next to this broken tissue there are several layers of cells (*a'*) which resemble those in fig. 16 immediately surrounding the sac. These are swollen and have every appearance of being active, nutritive cells. Beyond this again is the deeply staining tissue (*b*), but narrower now, as if some of the cells had changed in their appearance and had taken

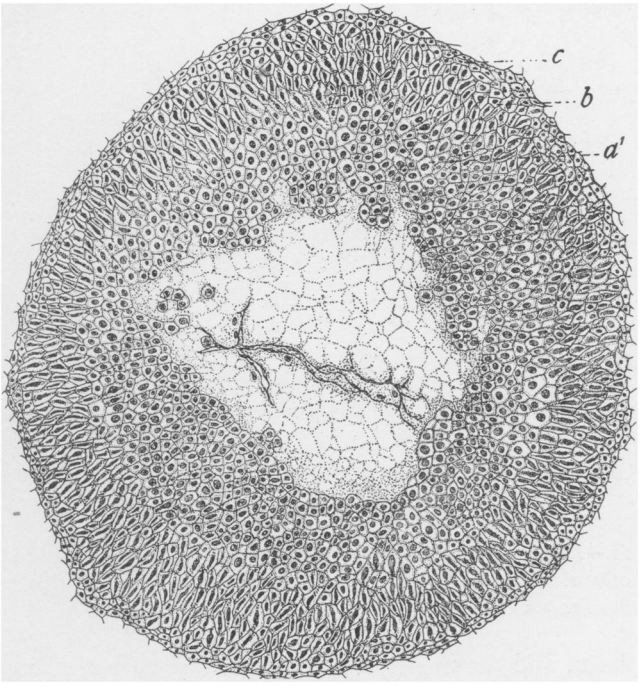


FIG. 21.—Detail of fig. 20. ×250.

on the function of the cells which previously bordered the sac. This tissue is narrower still toward the chalazal end of the sac.

Figs. 20 and 21 were made from an ovule in which the embryo sac was shrunken badly, but the amount of "spongy tissue" formed was unusually large and compact. The outer part shows radiation in its cell rows, as in other earlier stages. In one case this increase of "spongy tissue" was so great that it almost filled the interior, and the embryo sac had not developed. All these facts point to an active "spongy tissue" such as Miss FERGUSON claims for *Pinus* (**5**), and not

one which is formed early and which disappears early, leaving only a few thin layers, called "tapetum" by LANG (**3**) and not explained.

In fig. 22 this encroachment upon the "spongy tissue" has gone on until only about three or four rows are left between the sac and the bounding narrow cells (*c*). These inner cells have enlarged many times, as is shown by the magnification of the figures recorded. The walls of these cells (*b*) have become quite thick, the cells are full

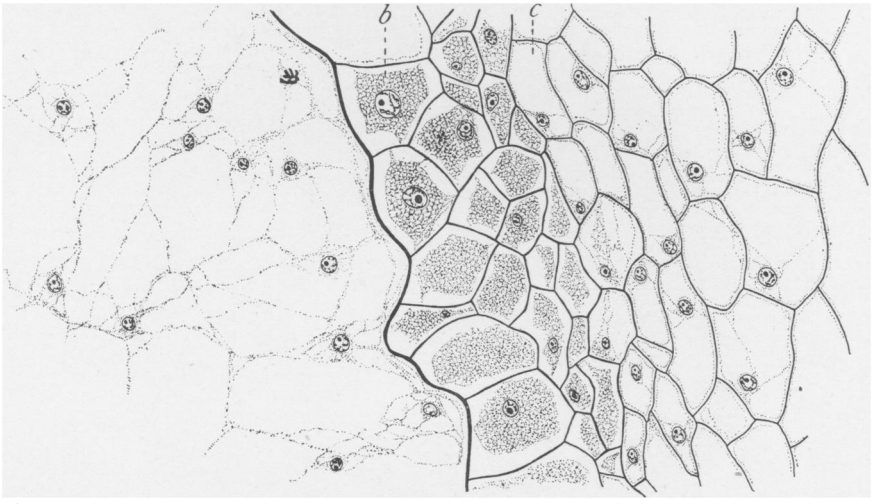


FIG. 22.—Detail of embryo sac with many free nuclei; diminished "spongy tissue"; cells of tissue (*b*) have increased in size and assumed nutritive function; *c*, flattened cells. ×580.

of cytoplasm, and the nuclei are not large, though not disorganized. The sac has now a broad cytoplasmic layer and a heavy wall. The nuclei are scattered everywhere, but have no walls as yet; at least none were seen in a careful examination with a $\frac{1}{12}$ oil immersion lens. The membrane is drawn away from the "spongy tissue" for a little distance at the micropylar end, so that in cutting the ovule it looks as if this end of the sac was not filled in.

From this stage on, the history of the development of the sac has been fully worked out in other forms by WARMING (**6**), whose description of the endosperm formation is excellent, by IKENO (**7**), and by others, so that it did not seem worth while to examine later material, at least not until the gaps behind could be closed up.

Summary

1. The young staminate and ovulate strobili can be distinguished by the difference in breadth, number of sporophylls, and number of meristematic points.

2. There is probably a single archesporial cell giving rise to a group of cells, one of which becomes the megaspore mother cell.

3. There are four potential megaspores, the lowest one becoming the embryo sac, whose development agrees with the accounts of other cycads in the main points.

4. The "spongy tissue" is an active, nutritive tissue, adding to its width by division of its cells as it is encroached upon by the embryo sac.

5. In its final degeneration, the cells of the "spongy tissue" nearest the embryo sac are first attacked, and the smaller cells outside them take their place, becoming large, swollen, and nutritive in function.

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LITERATURE CITED

1. SMITH, FRANCES GRACE, Morphology of the trunk and development of the microsporangium of cycads. BOT. GAZETTE **43**:187–204. *pl. 10.* 1907.
2. LANG, W. H., Studies in the development and morphology of cycadean sporangia. II. The ovule of *Stangeria paradoxa*. Annals of Botany **14**: 281–306. *pls. 17, 18.* 1900.
3. TREUB, M., Recherches sur les Cycadées. III. Embryogénie du *Cycas circinalis*. Ann. Jard. Buitenzorg **4**:1–11. *pls. 1–3.* 1884.
4. CHAMBERLAIN, C. J., The ovule and female gametophyte of *Dioon edule*. BOT. GAZETTE **42**:321–356. *pls. 13–15.* 1906.
5. FERGUSON, MARGARET, The spongy tissue of STRASBURGER. Science N.S. **18**:308–311. 1903.
6. WARMING, E., Undersogelser og Betragtninger over Cycadeerne. Oversight K. Vidensk. Selsk. Forh. pp. 100, 101. 1877.
7. IKENO, S., Untersuchungen über die Entwickelung der Geschlechtorgane und der Vorgang der Befruchtung bei *Cycas revoluta*. Jahrb. Wiss. Bot. **32**: 557–602. *pls. 8–10.* 1889.